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CLASSIFICATION AND REGRESSION TREE (CART) ANALYSIS FOR PREDICTING PROGNOSIS IN WILDLIFE REHABILITATION: A CASE STUDY OF LEPTOSPIROSIS IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract: The spirochete bacterium *Leptospira interrogans* serovar Pomona is enzootic to California sea lions (CSL; *Zalophus californianus*) and causes periodic epizootics. Leptospirosis in CSL is associated with a high fatality rate in rehabilitation. Evidence-based tools for estimating prognosis and guiding early euthanasia of animals with a low probability of survival are critical to reducing the severity and duration of animal suffering. Classification and Regression Tree (CART) analysis of clinical data was used to predict survival outcomes of CSL with leptospirosis in rehabilitation. Classification Tree outputs are binary decision trees that can be readily interpreted and applied by a clinician. Models were trained using data from cases treated from 2017-18 at The Marine Mammal Center in Sausalito, CA and tested against data from cases treated from 2010-12. Two separate Classification Tree analyses were performed, one including and one excluding data from euthanized animals. When data from natural deaths and euthanasias were included in model-building, the best Classification Tree predicted outcomes correctly for 84.7% of cases based on four variables: appetite over the first three days in care, and blood urea nitrogen (BUN), creatinine, and sodium at admission. When only natural deaths were included, the best model predicted outcomes correctly for 87.6% of cases based on BUN and creatinine at admission. This study illustrates that CART analysis can be successfully applied to wildlife in rehabilitation to establish evidence-based euthanasia criteria with the goal of minimizing animal suffering. In the context of a large epizootic that challenges the limits of a facility's capacity for care, the models can assist in maximizing allocation of resources to those animals with the highest predicted probability of survival. This technique may be a useful tool for other diseases seen in wildlife rehabilitation.

INTRODUCTION

The spirochete bacterium *Leptospira interrogans* serovar Pomona is endemic to California sea lions (CSL; *Zalophus californianus*) and causes periodic epizootics.[12] Clinical disease, i.e. leptospirosis, was first documented in cases admitted to California rehabilitation centers in 1970.[25] Historically, cases of leptospirosis have occurred yearly in the fall and these outbreaks exhibited a multi-annual cycle with larger epizootics occurring every three to five years.[9, 12] The largest number of leptospirosis related admissions to The Marine Mammal Center (TMMC) in Sausalito, CA, occurred in 2018 with over 300 cases (TMMC, unpubl. data). At the peak of the outbreak, more than 70 cases were admitted in a 30-day period. Clinical signs include polydipsia, lethargy, hyporexia to anorexia, melena, and apparent abdominal pain.[6, 9] Clinical pathology includes azotemia, hyperphosphatemia, hypernatremia, and variable leukocytosis. Leptospiral nephritis and associated comorbidities such as pneumonia and gastrointestinal ulceration are reported and can cause severe pain and discomfort. Reported case fatality rate in rehabilitation is 71%.[9]

Leptospirosis epizootics in CSLs, with their associated high morbidity, mortality and suffering, exemplify the acute need for evidence-based tools to effectively estimate prognosis for wildlife in rehabiliation. These tools are needed to establish data-driven euthanasia criteria to reduce suffering when prognosis is poor. Humane care and reduction of suffering are the clinician's highest priorities for an individual animal. Early identification of animals with a high probability of mortality is critical to reducing the duration and severity of pain and suffering. Financial and human resource limitations as well as hospital capacity must also be considered. These constraints may be particularly acute in facilities that rely heavily on volunteers and fiscal donations. In a facility with finite human and financial resources, individual animal care is likely

to decline as the hospital reaches and exceeds capacity. For example, increased infectious disease transmission, increased conspecific competition, decreased caregiver time available to each animal, and increased caregiver fatigue may occur. Therefore, a strategy of investing resources in those animals most likely to survive to release can result in reduced animal suffering and improved clinical outcomes, leading to overall greater rehabilitation success. Improved outcomes can be measured by, for example, improved response to treatment, reduced time in rehabilitation, lower prevalence of nosocomial infection, and lower incidence of secondary complications of captivity. Additional benefits can include greater financial sustainability, reduced caregiver burnout and compassion fatigue, and increased volunteer retention. All of these benefits feed back positively into the fundamental priorities of wildlife care and welfare.

While various statistical methods are available for modeling animal prognosis based on clinical data,[15] many of these methods are challenging to implement in clinical practice. Model outputs often require specialized statistical knowledge for interpretation, and input of new animal data for prognostic predictions requires specialized software and complex computation. Easy-to-use tools to link modeling analyses to clinical decisions, such as euthanasia, are lacking. This study describes the use of Classification and Regression Trees (CART) for predicting outcomes of California sea lions with leptospirosis in rehabilitation. Classification Tree outputs are simple binary decision trees that can be readily interpreted by a clinician and applied to predict case prognosis. This method has been used effectively for a variety of diagnostic and prognostic applications including in human cardiology,[7] oncology,[3, 24] and neurology,[13, 18, 21] and in veterinary equine and livestock medicine.[16, 19, 20, 23]

MATERIALS AND METHODS

Data from all CSL with leptospirosis admitted to TMMC in 2017-18 were evaluated (n = 356). All animals stranded along the Northern and Central California coast (approximate latitude range 34.373062 to 40.001243). Physical examination at admission was performed under manual or chemical restraint. Age class was determined based on straight total body length, dentition, and development of sagittal crest in males.[8] Age was classified as: pup (0-1 year), yearling (1-2 years), juvenile male (2-4 years), subadult male (4-8 years), adult male (8+ years), juvenile or subadult female (2-5 years), and adult female (5+ years).[8] Blood was collected from the caudal gluteal vein directly into vacutainer tubes (BD Vacutainer® SST™ and EDTA tubes, Oakville, Ontario LH6 6R5, Canada). Complete blood cell count was performed by Vet ABC Plus analyzer (SCIL Vet America, Gurnee, Illinois 60031, USA). White blood cell differentials were counted manually. Serum chemistry was performed by Axcel clinical chemistry analyzer (Alfa Wasserman-West, Caldwell, New Jersey 07006, USA). For clinical interpretation, blood values were compared to published ranges for wild, adult CSL[28] as well as in-house reference ranges generated using these analyzers and healthy, rehabilitated CSL at the time of release. A presumptive diagnosis of leptospirosis was based on clinical presentation, abdominal ultrasonography, and serum chemistry abnormalities including azotemia and electrolyte derangements.[9] Abdominal ultrasonography was used to screen for other common causes of azotemia including hydroureter and hydronephrosis secondary to urogenital carcinoma.[5] Multiple methods were used to confirm clinical diagnosis of leptospirosis. Serum antibodies to *L. interrogans* serovar Pomona were assayed by microscopic agglutination test (MAT; California Animal Health and Food Safety Laboratory, Davis, California 95616, USA) with a positive threshold of 1:3,200.[4] Leptospiral DNA presence in urine and kidney tissue was assessed using

real-time polymerase chain reaction (RT-PCR).[29] Gross necropsy and histopathology were utilized in animals that died.[9]

Treatment consisted of parenteral fluids, antimicrobials, gastroprotectants, and electrolyte supplementation. Animals were prescribed Lactated Ringer's Solution (LRS; Vetivex, Dechra Veterinary Products, Overland Park, Kansas 66211 USA; 100 mL/kg/day SQ for up to 10 days); actual dose and duration of administration varied by animal temperament. All animals received a single dose of ceftiofur (Excede, Zoetis, Parsippany, New Jersey 07054 USA; 6.6 mg/kg IM) upon admission. Oxytetracycline (LA-200, Zoetis; 20 mg/kg IM once every three days) was administered to animals that were not eating. While eating, animals received doxycycline (Epic Pharma, Laurelton NY 11413 USA; 5mg/kg PO BID) in herring. Tetracycline antibiotic therapy (oxytetracycline and/or doxycycline) was administered for a cumulative total of 14 consecutive days. Famotidine (Hikma, Eatontown, New Jersey 07724 USA; 1 mg/kg SQ, IM, or PO SID) was administered for three to seven days. In hypokalemic animals, potassium chloride (Pfizer, New York, New York 10017, USA) was supplemented in LRS to a total potassium content of 24 mEq/L. Animals received freshwater *ad libitum* and were offered frozen, thawed herring two to three times daily. When eating, animals received a multivitamin supplement in herring (Marine Mammal Supplement with Vitamin C, Mazuri, St. Louis, Missouri 63166 USA 1 tab PO SID).

To identify potential prognostic indicators, clinical data were investigated for associations with survival to release. Data from animals that survived were compared to those from all animals that died (natural death or euthanasia). Data for sex, age class, and all hematological and serum chemistry values at admission were fitted using logistic regression and tested for significance using likelihood ratio tests. Voluntary eating early in care was clinically observed to be associated with survival. To further investigate this trend, relative risk ratios

were calculated for animals that (1) ate voluntarily at least once in the first three days in care, (2) ate voluntarily at least once in the first seven days, (3) ate consistently within the first three days in care, and (4) ate consistently within the first seven days in care. Consistent eating was defined as eating at least once daily after the first instance of voluntary eating.

Candidate independent variables for prognostic modeling were selected based on the preliminary investigation of data. As the majority of deaths occurred on or after day four in care, and one of the goals of the model was to establish humane euthanasia criteria that reduced unnecessary stress and suffering, only variables that could be assessed prior to day four were considered. Of hematological and serum chemistry data, only variables relevant to the pathophysiology of leptospirosis and with significant associations to survival were considered as candidates. Candidate independent variables selected were age class, "appetite", and seven serum chemistry values at admission (blood urea nitrogen (BUN), creatinine, sodium, phosphorus, potassium, calcium, gamma-glutamyl transferase (GGT), and bilirubin). For the purpose of the model, the variable "appetite" was defined specifically as whether the animal ate voluntarily at least once during the first three days in care. To avoid confounding, only one of a set of physiologically correlated variables was included as a candidate; for example, sodium was included, and chloride excluded because these electrolyte concentrations are closely linked except in specific and relatively rare disease states. While BUN and creatinine are also physiologically linked, both were included as candidate variables because of their wider range of biological drivers including processes in the liver, muscle, and urinary and gastrointestinal systems.

CART analysis was used to evaluate the correlation between candidate independent variables and survival to release, and to create Classification Tree models.[2] Classification Trees

were built using binary recursive partitioning. Tree selection was done using 10-fold cross-validation of the training data, using the default method in the R package rpart[23] of selecting the simplest tree within one standard error of the minimum cost value.[2] Animals that were in care less than 24 hours at time of death (n = 6) were excluded from analysis as these animals had not yet received an examination nor admission bloodwork. If an animal was released and then readmitted to rehabilitation (n = 2), data from the second rehabilitation period were excluded. Animals with comorbidities were included (Table 1).

CART analyses were performed using two different definitions of the dependent variable, animal outcome. First, data from all deaths (natural and euthanasia) were included and compared to animals that survived to release. Second, animals that were euthanized were excluded, and data from animals that died naturally were compared to those that survived to release. To inform interpretation of these two different models, data from animals that did not survive were compared across type of death (died naturally or euthanized) for each candidate variable. Logistic regression curves were fit to the data and tested for significance with chi square analysis.

In addition to the original fit of models to 2017-18 training data by cross-validation to assess out-of-sample predictive ability, a further, more stringent out-of-sample analysis was conducted using test data from cases rehabilitated in prior years. In this analysis, predictive performance of the Classification Trees was assessed using test data from CSL with leptospirosis admitted to TMMC in 2010-12 (n = 188 cases for which adequate data were available; time period spans the most recent epidemic prior to 2017). This test dataset included cases that survived to release, died, and were euthanized. As the data were utilized to evaluate predictive performance of the Classification Trees, statistical investigation of associations between

individual clinical variables with survival was not performed. Medical care for 2010-12 cases was similar to that described for 2017-18 with one notable exception; a broader variety of antimicrobials were administered including (alone or in combination) penicillin G benzathine and procaine (Combi-Pen-48, Bimeda, Oakbrook Terrace, Illinois 60181, USA; 30,000 IU/kg IM once every 2 days), amoxicillin (Sandoz, Princeton New Jerysey, 08540, USA; 22 mg/kg PO BID), ceftiofur, doxycycline, and oxytetracycline. Dosing of ceftiofur and tetracyclines was the same as for 2017-18 cases.

To maximize confidence in variable selection and model performance, parallel analyses were conducted using logistic regression. Generalized linear models with logit link function and binomial error distribution were fitted to different combinations of variables with survival as a binary outcome variable. All combinations of candidate variables, with up to five in the same model, were used to fit models. Model performance was assessed by 10-fold cross-validation,[10] using deviance as the main statistic for model ranking and comparison.

Data manipulation, analysis, and plotting were done using R[17] and packages rpart,[22] ggplot2,[26] lme4,[1] and dplyr.[27]

RESULTS

Three hundred and fifty-six CSL with leptospirosis were admitted to TMMC for rehabilitation in 2017-18. Peak admissions occurred in August 2018. In animals that survived to release, clinical diagnosis was supported by MAT > 1:3,200 (n = 108) or MAT and RT-PCR positive urine (n = 40). MAT results ranged from 1:12,800 to 1:819,200 (mode 1:102,400). In animals that died (either natural death or euthanasia), clinical diagnosis was supported by gross necropsy (n = 48), necropsy and histopathology (n = 34), necropsy and RT-PCR positive urine or

kidney (n = 73), or necropsy, histopathology, and RT-PCR (n = 53). Age class distribution, voluntary eating, comorbidities, and time in care are presented in Table 1. Males made up 91.5% of cases, and juveniles were the most common age class admitted. Fifty-four percent of all deaths occurred on or after day four in care.

Clinical data including age, sex, appetite, and admission blood parameters for 2017-18 cases were investigated for associations with survival to release. Survival to release among juveniles (n = 198) was significantly higher than for all other age classes (adult n = 17, subadult n = 103, yearling n = 38, $P = 0.035$; Table 1). There was no significant difference in survival by sex (female n = 30, male n = 326, $P = 0.55$). Relative risk of death (either natural death or euthanasia) was significantly lower for animals that ate voluntarily at least once in the first three days in care (n = 152, RR = 0.31, 95% CI 0.24-0.41, $P < 0.0001$; Table 1) and those that ate at least once in the first seven days in care (n = 203, RR = 0.35, 95% CI 0.29-0.43, $P < 0.0001$) as compared to those that did not. Relative risk of death was also significantly lower for animals that ate consistently after first starting to eat voluntarily as compared to those that ate intermittently in the first three days (consistent eaters n = 127, RR = 0.20, 95% CI 0.23-0.41, $P < 0.0001$) and in the first seven days (consistent eaters n = 144, RR = 0.17, 95% CI 0.11-0.25, $P < 0.0001$). There were significant differences across outcomes for the following serum chemistry analytes: BUN, creatinine, sodium, phosphorus, potassium, calcium, bilirubin, and GGT ($P = 0.017$ for GGT, $P < 0.001$ for all other comparisons; Table 2). There were no significant differences across outcomes for complete blood count parameters.

The following candidate variables for prognostic modeling were selected: age class, appetite (voluntary eating within three days), and the seven serum chemistry values at admission (BUN, creatinine, sodium, phosphorus, potassium, calcium, GGT, and bilirubin) found to differ

significantly across outcomes. Data for each candidate variable was investigated for differences between animals that died naturally or were euthanized. Potassium was significantly higher in animals that died naturally as compared to those that were euthanized ($P < 0.0001$) while GGT was significantly lower ($P = 0.039$). There were no significant differences for appetite, age class, BUN, creatinine, sodium, phosphorus, calcium, and bilirubin.

Separate Classification Tree analyses were performed including and excluding data from euthanized animals. When all deaths (natural and euthanasia) were included, the best Classification Tree (henceforth "CT-All", Figure 1) had a predictive accuracy of 84.7% for the 2017-18 training data. CT-All included the variables BUN, creatinine, sodium, and appetite (voluntary eating within three days). When only natural deaths were included, the best Classification Tree ("CT-Nat", Figure 2) included only BUN and creatinine and had a predictive accuracy of 87.6%.

Data from 188 CSL with leptospirosis admitted to TMMC in 2010-12 were used as an independent dataset to evaluate the predictive performance of the Classification Trees trained using the 2017-18 dataset. In animals that survived to release, clinical diagnosis was supported by MAT > 1:3,200 (n = 39), RT-PCR positive urine (n = 5), or MAT and RT-PCR (n = 29). In animals that died (either natural death or euthanasia), clinical diagnosis was supported by MAT > 1:3,200 (n = 5), gross necropsy (n = 12), necropsy and MAT (n = 20), necropsy and histopathology (n = 2), necropsy and RT-PCR positive urine or kidney (n = 74), or necropsy, histopathology, and RT-PCR (n = 2). Overall mortality was 61.2% (26.6% died naturally and 34.6% were euthanized). Males made up 78.8% of cases. Age class distribution, voluntary eating, comorbidities, and time in care are presented in Table 1. Admission bloodwork is

presented in Table 3. When applied to the 2010-12 case dataset, CT-All had a predictive accuracy of 75.1% and CT-Nat had a predictive accuracy of 75.7%.

Parallel analyses using logistic regression were performed to compare to Classification Tree predictions. The top five logistic regression models for 2017-18 training data were ranked by cross-validation deviance (Table 4). Predictive accuracy for the top five models ranged from 80.3 to 83.6% for those including all deaths, and 85.5 to 89.8% for those including natural deaths only. All candidate variables included in the best Classification Trees (i.e. CT-All and CT-Nat) are present in one or more of the top-ranked logistic regression model(s). Appetite is present in all logistic regression models.

DISCUSSION

In this study, Classification Tree analysis was used to develop two prognostic models to assess survival probability of California sea lions in rehabilitation with leptospirosis. Both models had high predictive accuracy and required data readily obtained within the first three days of rehabilitation. Application of a Classification Tree using individual animal data to reach a predicted outcome is rapid and simple. Combined, these facts make the models excellent tools for evidence-based assessment of individual cases in rehabilitation. Use of the model-predicted outcomes to guide euthanasia decision-making will enable clinicians to relieve the suffering of animals unlikely to survive. In the context of a large epizootic that challenges the limits of a facility's capacity for care, the models can assist in maximizing efficient and effective allocation of resources to those animals with the highest predicted probability of survival to release. It is important to note that a model is never intended to replace individual clinician judgement and experience; rather it is an additional tool to enhance and inform clinical decision-making.

The use of CART analysis in epidemiology and medicine has been criticized as a "data mining" exercise in which all possible candidate variables are considered but the resulting clusters of clinical features in the CART output are not scrutinized for biological relevance.[10, 13] To address these concerns, this study utilized a limited number of biologically relevant candidate variables, relied heavily on out-of-sample tests, and performed parallel logistic regression analyses.

In clinical practice, either CT-All or CT-Nat is appropriate for assessment of, and euthanasia decision-making for, CSL with leptospirosis in rehabilitation. CT-All may be more valuable in clinical practice despite its slightly lower predictive performance due the inclusion of sodium at admission and appetite over the first three days in care. During the 2017-18 epidemic, clinicians observed repeated, intractable seizures in many severely hypernatremic animals; these animals were euthanized on humane grounds but would likely have otherwise died naturally. Similarly, animals with prolonged anorexia and clinical signs of cachexia were euthanized on humane grounds; these animals also would likely have died naturally if not euthanized. Therefore, the application of euthanasia criteria based on CT-All may facilitate identification and earlier euthanasia of animals that would otherwise suffer from these conditions. This is supported by the logistic regression analysis; appetite was present in all ten top-ranked models, and sodium was present in five (Table 4).

CT-Nat is an excellent alternative to employ in clinical practice. This model has slightly higher predictive performance, requires fewer variables, and was developed using a dataset which included only deaths that occurred naturally. Although there were no significant differences across type of death (natural or euthanasia) in the four predictive variables used by CT-All, exclusion of data from euthanized animals in CT-Nat eliminates uncertainty regarding

whether those animals could ultimately have survived. Use of CT-Nat is recommended if a clinician does not have access to data for sodium at admission or appetite over the first three days in care, or if a clinician wishes to eliminate any possible prognostic bias against animals that might have survived without the intervention of euthanasia. If CT-Nat is used, seizure and prolonged anorexia should be considered as grounds for humane euthanasia apart from model predictions. For clarity and ease of use, the use of one model or the other is suggested for a single rehabilitation facility or case population.

By design, the models presented here do not consider variation in comorbidities. Comorbid conditions range widely in type and severity, may be difficult to detect ante-mortem, and have variable impacts across individuals. Therefore, inclusion of all cases, regardless of comorbidities, in model development improves the utility of the resulting decision tree in clinical practice. Theoretically it would be possible to add comorbidities as a covariate in the models, but this would add complications to clinical application, and developing a robust model of this type would require a larger sample size than is currently available. Despite the wide range of comorbidities in both the training and test datasets, the overall predictive performance of the models presented here is strong, predicting outcome correctly in 84.7 to 87.6% of cases. By comparison, predictive performance of CART models found in human and veterinary literature ranged from 77 to 94.5%.[3, 13, 18, 20, 21] However, survival of animals with severe comorbidities may be overestimated by the model; thus clinical judgement should be employed in application of the decision tree to these cases.

Although lower than for 2017-18 data, the predictive performance of the Classification Trees for 2010-12 case data is strong. This demonstrates that the models are robust to variations in population attributes such as treatment protocols and comorbidity prevalence. For example,

2010-12 cases had a notably higher prevalence of malnutrition (Table 1) and were treated with a wider range of antimicrobials. The difference in predictive performance does highlight that the models are strongest when applied to cases that have experienced similar external factors including environmental conditions and medical treatments. In addition, periodic retraining of the model using larger datasets may be valuable in improving predictive accuracy. The use of a different clinical chemistry analyzer as compared to that used in this study may affect measured values and should be taken into consideration when utilizing the decision trees.

Comparison of CART with logistic regression analysis of the same data increased confidence in variable selection and model performance. Predictive accuracy of the top ranked logistic regression models for 2017-18 cases ranged from 80.3 to 89.8% (Table 4); this is similar to the performance of the Classification Trees. All variables in Classification Trees were present in one or more top ranked logistic regression model(s). Interestingly, potassium was present in all but one of the top ranked logistic regression models but not in the Classification Trees. In contrast, BUN is absent from seven of ten top ranked logistic regression models yet included in the Classification Trees. By design, these two modeling approaches respond differently to predictor variables (and interactions among them), and the underlying linearity assumed by the logistic regression model may be excluding useful information in the BUN data. Further investigation into the prognostic value of these variables using data from future cases may be indicated.

In wildlife rehabilitation, evidence-based prognostic tools and euthanasia criteria can enhance humane animal care and facilitate allocation of resources towards individuals most likely to survive. Such tools are particularly important for diseases such as leptospirosis that have a high potential for pain and suffering, prolonged course of illness prior to death, and

resource-intensive treatment protocols. The need for such tools is amplified in the case of a large outbreak in which the physical, personnel and fiscal capacities of a facility may be pushed to their limits. Euthanasia criteria are most useful if based on data that can be acquired readily and early in treatment and can be applied early enough to reduce the duration of suffering of a terminally ill animal.

In contrast to a variety of other statistical methods available for predicting prognosis, Classification Trees offer an intuitive, clinician-friendly output that does not require computation nor specialized knowledge for application.[11, 15] This study has shown that CART analysis can be successfully applied to wildlife in rehabilitation and can predict survival with a high degree of accuracy. This technique may be a useful means of predicting prognosis for other diseases seen in wildlife rehabilitation, particularly those for which there is abundant historical clinical data available for analysis. Models may be most useful for diseases that are endemic, that cause large epidemics, or for mass mortality events. Examples include care of wildlife affected by oil spills, harmful algal blooms and other biotoxins, morbillivirus epidemics, epidemic pasteurellosis, and botulism.

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Table 1. Age class, voluntary eating, comorbidities and time in care for California sea lions with leptospirosis admitted to The Marine Mammal Center in 2017-18 (n = 356) and 2010-12 (n = 182). Values for age class, appetite, and comorbidities are reported by outcome (released, died naturally, or euthanized) and as the number and proportion (e.g., n (n/total)) of animals within each outcome category (released, died naturally, or euthanized) for each sample group (2017-18 or 2010-12). Appetite data is presented for the first three days in care, with consistent eating defined as voluntary eating at least once daily after the first instance. Comorbidity diagnosis was based on gross necropsy (all animals that died) and physical examination (all released animals). Time in care is reported as the number of days in care prior to disposition (i.e., release or death). Mean and range are shown by outcome for each sample group.

	2017-2018 Cases			**2010-2012 Cases**		
	Released	**Died Naturally**	**Euthanized**	**Released**	**Died Naturally**	**Euthanized**
	n=148	*n=94*	*n=114*	*n=73*	*n=50*	*n=65*
Age Class						
Adult	4 (0.03)	5 (0.05)	8 (0.07)	4 (0.05)	4 (0.08)	4 (0.06)
Subadult	36 (0.24)	33 (0.35)	34 (0.30)	13 (0.18)	13 (0.26)	13 (0.20)
Juvenile	96 (0.65)	50 (0.53)	52 (0.46)	48 (0.66)	25 (0.50)	34 (0.52)
Yearling	12 (0.08)	6 (0.06)	20 (0.18)	8 (0.11)	8 (0.16)	14 (0.22)
Appetite						
Ate At Least Once	113 (0.76)	15 (0.16)	24 (0.21)	61 (0.84)	22 (0.44)	24 (0.37)
Ate Consistently	106 (0.72)	10 (0.11)	11 (0.10)	54 (0;74)	9 (0.18)	12 (0.18)
Comorbid Disease						
Malnutrition	123 (0.83)	65 (0.69)	78 (0.68)	49 (0.67)	49 (0.82)	59 (0.98)[c]
Gastrointestinal ulceration[a]	-	63 (0.07)	54 (0.47)	-	15 (0.25)	20 (0.33)[c]
Pneumonia[a]	-	46 (0.49)	44 (0.39)	-	12 (0.20)	7 (0.12)[c]
Abscess[b]	8 (0.05)	15 (0.16)	15 (0.13)	3 (0.04)	8 (0.13)	8 (0.13)[c]
Major trauma	2 (0.01)	5 (0.05)	16 (0.14)	0 (0)	0 (0)	4 (0.07)[c]
Urogenital carcinoma[a]	-	3 (0.03)	1 (0.01)	-	0 (0)	1 (0.02)[c]
Polyphasic rhabdomyositis	0 (0)	0 (0)	1 (0.01)	0 (0)	0 (0)	1 (0.02)[c]
None documented	16 (0.11)	4 (0.04)	4 (0.04)	22 (0.30)	1 (0.02)	2 (0.03)[c]
Days in Care						
Mean (Range)	25 (18-57)	4 (0-36)	7 (0-39)	31 (11-92)	4 (0-16)	5 (0-34)

[a] Data reported from 60 animals for which gross necropsy was performed; 5 animals from the sample population that were euthanized were not necropsied.

[b] Comorbidity prevalence not reported for released animals due to low diagnostic sensitivity of physical examination alone, and inconsistent use of advanced diagnostics required for confirmation of antemortem diagnosis (e.g., radiography, bronchoscopy, endoscopy).

[c] Abscess sites include subcutis, muscle, and lymph node.

Table 2. Select hematology and serum chemistry data for California sea lions with leptospirosis admitted to The Marine Mammal Center in 2017-18 (n = 356). Data are from blood samples collected within the first three days in care. Mean and range are shown for animals that survived to release, died naturally, and were euthanized.

Analyte[a]		Reference Interval[27]	Released *n=148*		Died Naturally *n=94*		Euthanized *n=114*	
			Mean	Range	Mean	Range	Mean	Range
WBC	$10^3/\mu L$	9.4 - 22.8	24.5	5.8 - 60.7	20.4	5.8 - 60.7	24.1	2.0 - 54.0
RBC	$10^6/\mu L$	4.06 - 4.29	4.4	1.7 - 5.9	4.6	3.4 - 7.2	4.4	2.7 - 7.2
Hemoglobin	g/dL	15.0 - 15.9	16.1	10.0 - 23.0	17	2.7 - 12.5	16.2	11.2 - 26.4
Hematocrit	%	ND	44.9	21.0 - 64.8	48	34.3 - 79.4	46	31.3 - 76.7
BUN	mg/dL	30 - 38	154.3	21.0 - 443.0	304.1	18.0 - 448.0	273.4	54.0 - 552.0
Creatinine	mg/dL	0.9 - 1.1	3.8	0.9 - 15.9	9	1.0 - 32.7	8.7	1.7 - 21.4
Phosphorus	mg/dL	6.7 - 7.2	9.9	4.9 - 17.0	15.2	4.9 - 38.5	14.7	6.1 - 28.1
Potassium	mmol/L	4.5 - 4.7	3.5	2.1 - 4.9	6.1	2.4 - 19.8	4.3	2.4 - 19.8
Sodium	mmol/L	151 - 152	160.3	141.3 - 198.4	168.6	142.2 - 203.8	168.1	140.2 - 203.6
Chloride	mmol/L	109 - 111	120.5	90.3 - 155.2	131.4	101.3 - 172.8	128.4	99.5 - 186.8
Calcium	mmol/L	9.5 - 9.7	9.4	7.7 - 10.8	8.5	6.4 - 10.9	8.7	5.3 - 11.0
AST	U/L	32 - 45	29.3	0 - 489.0	130.5	0 - 4743.0	68.3	0 - 673.0
ALT	U/L	35 - 47	34.4	1.0 - 231.0	53.3	1.0 - 1041.0	274.4	0 - 21660.0
ALP	U/L	76 - 96	93.8	13.0 - 6795.0	55.8	23.0 - 127.0	60.6	23.0 - 635.0
GGT	U/L	56 - 79	335.4	78.0 - 1023.0	338.6	0 - 1229.0	417.4	8.0 - 1231.0
Bilirubin	mg/dL	02. - 0.3	0.7	0.3 - 2.9	1.2	0.4 - 3.7	1.3	0.3 - 13.8

[a] WBC indicates white blood cells; RBC, red blood cells; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; ND, no data available. Reference interval data from adult, wild California sea lions.[28]

Table 3. Select hematology and serum chemistry data for California sea lions with leptospirosis admitted to The Marine Mammal Center in 2010-12 (n = 188). Data are from blood samples collected within the first three days in care. Mean and range are shown for animals that survived to release, died naturally, and were euthanized. In contrast to 2017-18 cases, gamma-glutamyl transferase (GGT) was not measured 2010-12.

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Analyte[a]		Reference Interval[27]	Released *n=73*		Died Naturally *n=50*		Euthanized *n=65*	
			Mean	Range	Mean	Range	Mean	Range
WBC	$10^3/\mu L$	9.4 - 22.8	25.5	7.4 - 51.6	25.2	8.7 - 55.0	24.3	3.6 - 67.8
RBC	$10^6/\mu L$	4.06 - 4.29	4.5	3.34 - 5.55	4.57	3.07 - 5.75	4.8	2.22 - 5.93
Hemoglobin	g/dL	15.0 - 15.9	16.7	11 - 19	15.9	10.5 - 20.4	16.6	6.8 - 21.4
Hematocrit	%	ND	46.6	32.9 - 59	47.7	31.7 - 62.3	50.6	21.7 - 65.3
BUN	mg/dL	30 - 38	140.9	41 - 447	262.6	67 - 522	258.6	23 - 804
Creatinine	mg/dL	0.9 - 1.1	2.9	0.9 - 13.7	6.2	0.4 - 16.8	5.1	0.36 - 12.7
Phosphorus	mg/dL	6.7 - 7.2	8.6	4.3 - 19.8	15.1	4.9 - 36.5	12.8	4.2 - 27.7
Potassium	mmol/L	4.5 - 4.7	3.7	2.6 - 4.9	4.8	2.7 - 15.8	4.3	2.8 - 7.7
Sodium	mmol/L	151 - 152	160.1	141 - 201	174.0	146 - 214	176.5	135 - 216
Chloride	mmol/L	109 - 111	122.7	105 - 148	134.2	108 - 171	136.3	95 - 182
Calcium	mmol/L	9.5 - 9.7	8.8	6.7 - 10.4	8.2	5.5 - 10.2	8.3	5.7 - 10.1
AST	U/L	32 - 45	37.7	9 - 134	90.4	8 - 987	64.5	10 - 316
ALT	U/L	35 - 47	56.5	20 - 203	66.1	24 - 206	65.6	19 - 265
ALP	U/L	76 - 96	60.9	16 - 366	51.5	23 - 102	99.3	23 - 1991
Bilirubin	mg/dL	02. - 0.3	0.7	0.2 - 2.6	1.6	0.3 - 8.2	1.4	0.3 - 8.1

[a] WBC indicates white blood cells; RBC, red blood cells; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ND, no data available. Reference intervals from adult, wild California sea lions.[28]

Table 4. Logistic regression models predicting outcome of California sea lions with leptospirosis in rehabilitation at The Marine Mammal Center in 2017-18. The top five best-fit models are shown for data including all deaths (natural and euthanasia) and natural deaths only. Models were limited to five variables maximum and are ranked by ten-fold cross-validation deviance. The candidate variables included in each model are indicated by a check mark.

Model		Predictive Accuracy (%)	Cross-Validation Deviance	Candidate Variables[a]									
				Appetite	Potassium	Creatinine	Sodium	Phosphorus	Age Class	Calcium	Bilirubin	BUN	GGT
Inclusive of all deaths (natural & euthanasia)	A	81.4	220.1	√	√	√			√			√	
	B	82.5	221.2	√	√	√	√	√					
	C	80.3	221.7	√	√	√			√		√		
	D	83.6	223.5	√	√	√	√				√		
	E	81.8	224.0	√	√	√			√				
Inclusive of natural deaths only	F	87.1	124.7	√	√	√	√					√	
	G	85.5	126.1	√		√	√				√		√
	H	88.2	126.9	√	√	√		√		√			
	I	87.6	126.9	√	√		√	√		√			
	J	89.8	127.0	√	√			√		√		√	

[a] BUN indicates blood urea nitrogen; GGT, gamma-glutamyl transferase; ND, no data available.

Figure 1. Classification Tree ("CT-All") for prognosis of California sea lions admitted to The Marine Mammal Center and diagnosed with leptospirosis. The tree was fitted to a training dataset consisting of cases from 2017-18 (inclusive of animals that died naturally and were euthanized) and tested against a dataset of cases from 2010-12 (also inclusive of all causes of death). Serum chemistry values are from a blood sample collected within three days of admission to rehabilitation. Appetite is assessed by whether an animal eats voluntarily at least once within three days of admission to rehabilitation. Decision criteria are shown in unshaded oval nodes. Model predictions of death or survival are shown in unshaded rectangles. Observed outcomes of individuals in the training dataset (2017-18) are shown in shaded rectangles. To apply this Classification Tree to an individual case, begin at the first oval node "BUN $\geq$ 280 mg/dL". If this statement is true, follow the branch to the left to find that the model predicts death. If false, follow the branch to the right and continue to apply the decision criteria in each oval node until arriving at the model prediction of survival or death. For example, consider an animal with the following clinical data: BUN 171 mg/dL, creatinine 4.4 mg/dL, sodium 165.8 mmol/L, and ate once on day two in care. The model predicts that this animal will survive.

Figure 2. Classification Tree ("CT-Nat") for prognosis of California sea lions admitted to The Marine Mammal Center and diagnosed with leptospirosis. The tree was fitted to a training dataset consisting of cases from 2017-18 that survived to release or died naturally (exclusive of animals that were euthanized) and tested against a dataset of cases from 2010-12 (inclusive of all causes of death). Serum chemistry values are from a blood sample collected within three days of admission to rehabilitation. Decision criteria are shown in unshaded oval nodes. Model predictions of death or survival are shown in unshaded rectangles. Observed outcomes of

individuals in the training dataset (2017-18) are shown in shaded rectangles. To apply this Classification Tree to an individual case, begin at the first oval node "Creatinine ≥ 7.9 mg/dL". If this statement is true, follow the branch to the left to find that the model predicts death. If false, follow the branch to the right and continue to apply the decision criteria in the oval node to arrive at the model prediction of survival or death. For example, consider an animal with the following clinical data: BUN 120 mg/dL and creatinine 1.2 mg/dL. The model predicts that this animal will survive.